STRUCTURAL FEATURES OF ZINC AND SILICATE CO-SUBSTITUTED HYDROXYAPATITE AS BIOMATERIAL FOR MEDICINE

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Abstract

The hydroxyapatite is a material chemically similar to the mineral composition of human bone and dental tissues. The introduction of substituents into the structure of HA leads to changes in the biological and physico-chemical properties of materials based on such substitution. The samples with different equal concentration of substituents such as zinc and silicate were obtained via the mechanochemical synthesis. The co-substituted HA samples were investigated by means of FTIR spectroscopy and X-ray powder diffraction. It is shown that this method of the synthesis makes it possible to obtain a single-phase product after 30 min of mechanical action in a planetary ball mill. The substitution limit for the case of double equal substitution has been determined. The effect of heat treatment on the structure of synthesized materials is revealed.

Keywords: *hydroxyapatite, co-substitution, zinc, silicate*

1. INTRODUCTION

The artificial hydroxyapatite (HA) – $Ca_{10}(PO_4)_6(OH)_2$ – is similar in chemical composition to the mineral component of a natural bone, due to HA is widely used in medicine as a material for filling bone defects and for coating implants [1]. Despite the fact that HA has the ability to bind with bone tissue, HA has a lower biological activity compared to other calcium phosphates [2]. However, it is known that the introduction of various substituent ions into the structure of apatite can not only increase its activity, but also give other useful properties [3].

The HA structure has a space group of symmetry $P6_3/m$ with the unit cell parameters *a* and *c* equal to 9.432 Å and 6.881 Å, respectively. It is well known that the HA unit cell contains 10 calcium cations, 6 tetrahedra of phosphate groups, and 2 hydroxyl groups forming a channel on the *c* axis. The calcium cations occupy two different positions: the CaI and CaII sites. The site, in which the one calcium cation is surrounded by nine oxygen of phosphate groups, is referred to as the CaI site. The site, in which the one calcium cation is surrounded by six oxygen of phosphate groups and the one oxygen of hydroxyl group, is referred to as the CaI site. This structure of HA allows to carried out the both anionic and cationic co-substitution in the HA structure, which affect the physicochemical and biological properties of the ceramics [3-4].

It is known that the incorporation of a silicate group into the HA structure, with the formation of a silicate-substituted HA (HA-Si), where the silicate group replaces the phosphate group, enhances the processes of HA solubility and the formation of new bone tissue [5]. It is important that the limit of substitution of phosphate for silicate into the HA depends on the its method of synthesis. Bulina et al. found that the methods that do not require high-temperature treatment allow the introduction of up to 2 mol of silicon per 1 mol of HA [6], however the use of additional annealing leads to a decrease in the substitution limit since at the temperatures more than 970 °C and a silicon concentration of more than 1 mol per 1 mol of HA the impurity phase of $Ca_5(PO_4)_2SiO_4$ is formed [7]. When the degree of substitution of phosphate for silicate is not more than 0.6 mol this transition is not observed, such a compound is stable up to the temperature of 1200 °C [7].

An example of cationic substitution in the HA lattice is the substitution of calcium ions for zinc ions [8]. It is known that the incorporation of zinc cations into the HA structure gives the material antibacterial properties [9]. Depending on the conditions for the synthesis of zinc-substituted HA (HA-Zn), the position of zinc in the structure of HA can change. If the Zn-HA is obtained by the

mechanochemical synthesis [10-11] or the precipitation method [12-13], the zinc ions occupy the positions of calcium cations. Miyaji et al. showed that the substitution limit in this case is ~1.5 mol [14]. The high-temperature treatment during synthesis leads to the displacement of zinc ions from calcium site into the hexagonal channel, where they replace the OH groups with the formation of O–Zn–O channel [8, 10-11, 15]. In this case there is a significant reduction in the substitution limit – 0.3 mol Zn per 1 mol of HA [8].

Friederichs et al. showed that the HA can be synthesized with the simultaneous co-substitution of calcium for zinc and phosphate for silicate (HA-ZnSi) by precipitation method with further annealing at 1100 ^{o}C [16]. The authors obtained the single phase samples with a concentration of zinc up to 0.1 mol and silicon up to 0.5 mol per mol HA. Based on the DFT modelling the authors assumed that the zinc ion probably occupies an interstitial position just off the *c*-axis near a silicate anion.

The aim of this work is to investigate the effect of substituent ions on the structure of the zinc and silicate co-substituted HA obtained via mechanochemical synthesis and to determine the limit of co-substitution.

2. MATERIALS AND METHODS

2.1. Mechanochemical synthesis

The series of HA-ZnSi samples was obtained by mechanochemical synthesis in the AGO-2 planetary ball mill. The synthesis was carried out into the water-cooled steel vials with the 0.8 cm steel balls at the rotation speed of the vials 1200 rpm during 30 min. The ratio of the weight of the reaction mixture to the mass of the balls was 1:20. To prevent contamination of the product, the vials were lined with a reaction mixture for 0.5 min.

The initial reagents were the freshly calcined calcium oxide CaO, the calcium hydrophosphate $CaHPO_4$, the amorphous silicon oxide $SiO_2{\cdot}nH_2O$ and the zinc dihydrophosphate $Zn(H_2PO_4)_2{\cdot}2H_2O$. The ratio of reagents was taken in accordance with the equations given in Tab. 1.

Sample notation	Ratio of reactants	Expected composition
HA-0.0ZnSi	$6.0CaHPO_4 + 4.0CaO$	$Ca_{10}(PO_4)_6(OH)_2$
HA-0.2ZnSi	$5.4CaHPO_4 + 4.4CaO + 0.2Zn(H_2PO_4)_2{\cdot}2H_2O + 0.2SiO_2{\cdot}nH_2O$	$Ca_{9.8}Zn_{0.2}(PO_4)_{5.8}(SiO_4)_{0.2}(OH)_{1.8}$
HA-0.6ZnSi	$4.2CaHPO_4 + 5.2CaO + 0.6Zn(H_2PO_4)_2{\cdot}2H_2O + 0.6SiO_2{\cdot}nH_2O$	$Ca_{9.4}Zn_{0.6}(PO_4)_{5.4}(SiO_4)_{0.6}(OH)_{1.4}$
HA-1.0ZnSi	$3.0CaHPO_4 + 6.0CaO + 1.0Zn(H_2PO_4)_2{\cdot}2H_2O + 1.0SiO_2{\cdot}nH_2O$	$Ca_{9.0}Zn_{1.0}(PO_4)_{5.0}(SiO_4)_{1.0}(OH)_{1.0}$
HA-2.0ZnSi	$8.0CaO + 2.0Zn(H_2PO_4)_2{\cdot}2H_2O + 2.0SiO_2{\cdot}nH_2O$	$Ca_{8.0}Zn_{2.0}(PO_4)_{4.0}(SiO_4)_{2.0}$

Table 1. Ratio of the initial reagents and expected product of the mechanochemical synthesis

In order to refine the structure of the co-substituted apatite the synthesized HA-Zn-Si were heated in the high-temperature chamber electrical furnace (PVK 1.4-8) at 300, 650 and 750 °C for 2 h.

2.2. Methods of analysis

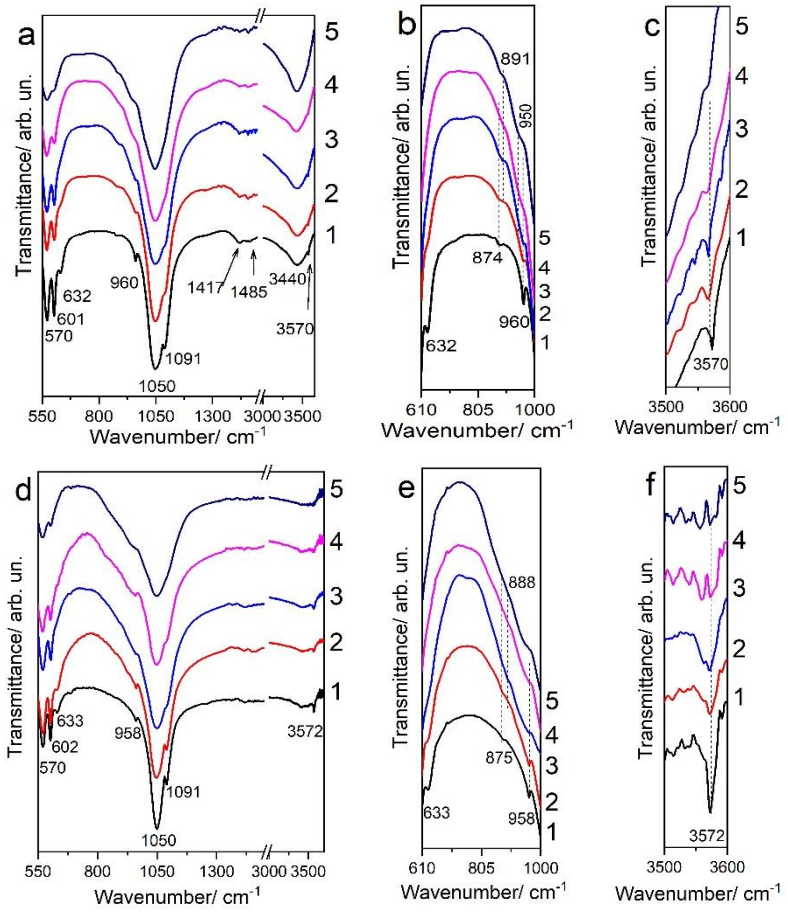
FTIR spectra were recorded with the help of an Infralum-801 spectrometer (Simex, Russia). The specimens were prepared by a KBr pellet method.

XRD patterns were recorded on a Bruker D8 Advance powder diffractometer in Bragg-Brentano geometry with CuK_α radiation. X-ray phase analysis of the compounds was carried out using the database of powder XRD patterns ICDD PDF-4 (2011). The unit cell parameters and crystallite size, as well as the amorphous phase concentration were determined by the Rietveld method in the Topas 4.2 program (Bruker, Germany).

3. RESULTS AND DISCUSSION

3.1. FTIR spectroscopy

Figure 1 a shows FTIR spectra of the pure HA and the zinc and silicate co-substituted HA. The major asymmetric streching modes P–O bond of PO_4^{3-} group were detected at wavelengths 1050 and 1091 cm^{-1}. The symmetric streching mode P–O was observed at 960 cm^{-1}. The modes at 960 and 1091 cm^{-1} are disappeared as the amount of zinc and silicate doping increased. The intensity of absorption bands at wavelengths 570 and 601 cm^{-1} of the bending modes of PO_4^{3-} group decreases with the increasing of substitution degree. The intensity of the absorption bands at 632 and 3570 cm^{-1}, belonging to the libration and symmetric stretching modes of the OH-group, respectively, decreases with increasing concentration of the introduced dopants (Fig. 1 b, c). Furthemore, these bands are absent in the spectrum of HA-2.0ZnSi sample, which corresponds to the assumed chemical formula of the HA-2.0ZnSi sample. In addition, the spectra of co-substituted HA have the absorption bands at a wavelength of 891 and 950 cm^{-1}. These bands was detected in the FTIR spectra of HA-Si samples and attributed to the silicate ion [6]. The wide absorption bands at the wavelengths of 875, 1417, 1485, and 3440 cm^{-1} were associated with vibrations in the adsorbed carbon dioxide and water molecules that were absorbed from the air atmosphere during the mechanochemical synthesis.



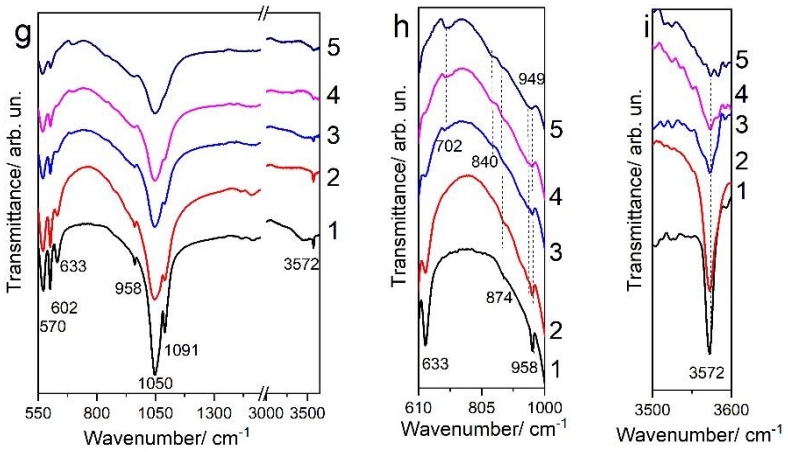


Fig. 1. FTIR spectra of as-synthesized HA-ZnSi samples (a-c), samples heated to 300°C (d-f) and at 750°C (g-i): 1 – HA-0.0ZnSi; 2 – HA-0.2ZnSi; 3 – HA-0.6ZnSi; 4 – HA-1.0ZnSi; 5 – HA-2.0ZnSi

Heating the samples to a temperature of 300 °C leds to the removal of adsorbed carbonate ion and water (Fig. 1 d-f). In addition, the absorption bands of OH groups at 633 and 3572 cm^{-1} became more intense. The new band at wavelength of 702 cm^{-1} appeared in the spectrum of HA-2.0ZnSi sample after heating at 750 °C (Fig. 1 h). This band may be due to the appearance of a new hydrogen bond with a substituent, possibly a zinc ion.

3.2. XRD analysis

Figure 2 shows the XRD patterns of as-synthesaized materials. All the pattetns contain one crystalline phase, namely HA phase (PDF [040-10-6315]). It is seen that there is a decrease in the intensity of the reflections with an increase in the concentration of the introduced ions accompanied by an apearanse of large amount of an amorphouse phase in the HA-2.0ZnSi sample. This indicates that the introduced dopants reduces the crystallinity of HA. Because the FTIR spectrum of HA-2.0ZnSi sample does not differ from one of other co-substituted samples, it can be assumed that the amorphous phase is nanosized particles of the co-substituted HA-ZnSi. A drop in the intensity of the reflection (100) conferms the decrease of OH-groups amount in the co-substituted samples.

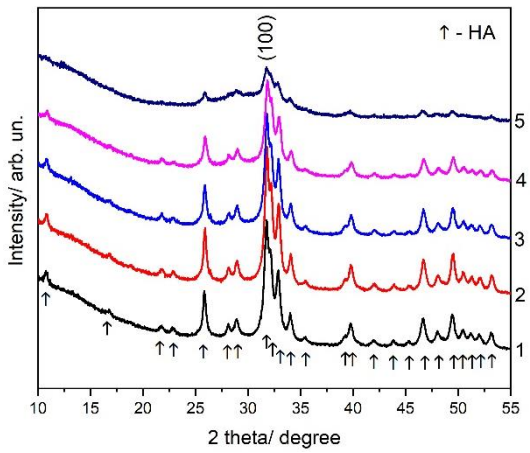


Fig. 2. XRD patterns of the as-synthesized HA-ZnSi samples: 1 – HA-0.0ZnSi; 2 – HA-0.2ZnSi; 3 – HA-0.6ZnSi; 4 – HA-1.0ZnSi; 5 – HA-2.0ZnSi

The refinement of the structural parameters for the as-synthesized samples by the Rietveld method shows that the change in the lattice parameters *a* and *c* have a nonlinear dependency (Fig. 3 a,b). There is a smooth decrease in the parameters *a* and *c* upto the substituents consentration x = 0.6, and then the parameters increase. As our previous studies have shown, in the samples obtained by the mechanochemical method with a single substitution of zinc ions for calcium ions, a decrease in the both parameters is observed with an increase in the zinc concentration, which is explained by the smaller diameter of the substituent ion [10, 17]. When the phosphate ion is replaced by the silicate ion in terms of the mechanochemical synthesis, a decrease in the parameter *a* and an increase in the parameter *c* is observed for the consentrations upto 0.6 mol. With a further increase in the concentration of a silicate, the changes become ambiguous [6]. It should be noted that the Zn-substitution most significantly infuence on the parameter *c*, while the parameter *a* is most significantly affected by the introduction of a silicate ion. Based on these data it can be concluded for the case of duble substitution that the decrease of the *a* and *c* parameter for low degree of co-substitution is the influence of the both introduced ions. An increase in the parameters at a concentration of more than 0.6 mol can be associated with an increasing concentration of the crystallization water introdused to the reaction mixture by the amorphous silicon oxide (see Table 1). Indeed, in samples heated to 300°C, no increase in the parameters for this region of substituant concentration is observed (Fig. 3 a,b). However, a heating to higher temperatures gives a linear increase in the parameter *c* over the entire concentration range. Obviously, this is due to a structural changes in the substituted apatite. One of the options is the movement of the zinc ion to the most energetically favourable interstitial site near the *c*-axis and the silicate tetrahedron, which was suggested by Friederichs et al. based on DFT modelling [16].

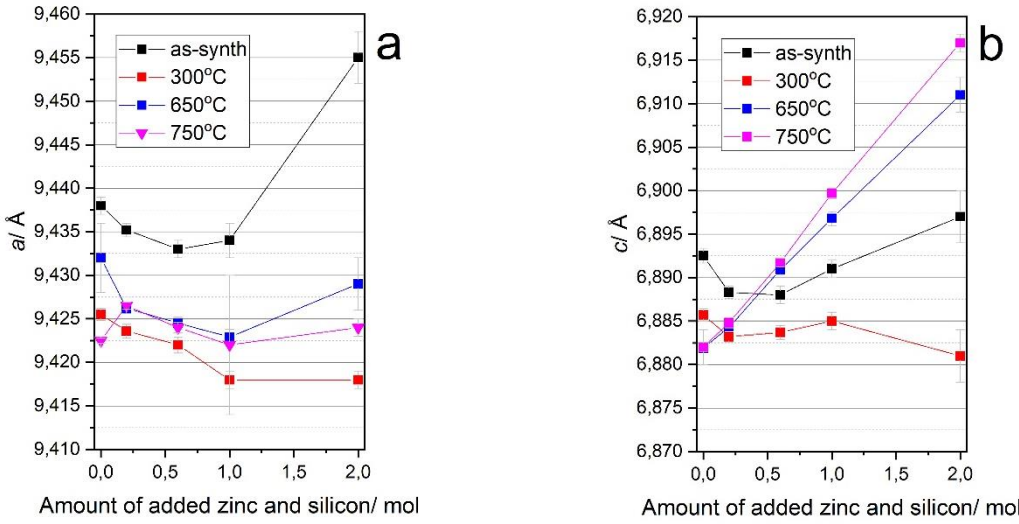


Fig. 3. The evolution of the unit cell parameters *a* (a) and *c* (b) as a function of the amount of the added substituents for the as-synthesized and heated samples

The investigation of the phase composition of the samples after heating showed low thermal stability of the sample HA-2.0ZnSi (Fig 4). Unlike the rest of the samples, the phase of ZnO appear in this sample after 750°C. This observations as well as the presense of essential amount of amorphouse phase in the sample at the as-synthesized state point out on the limit of co-substitution is less than 2 mol of the silicon and zinc per mol of HA.

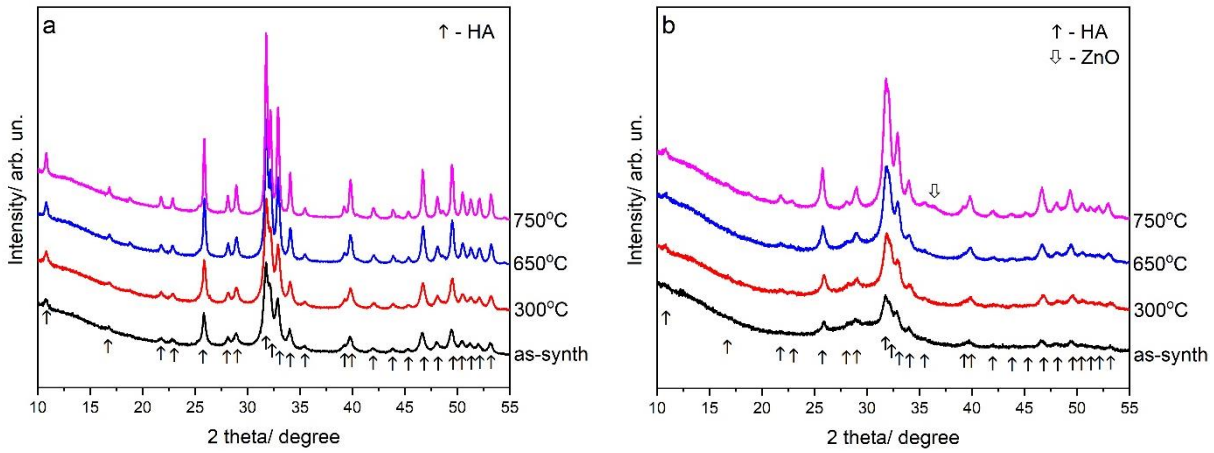


Fig. 4. XRD patterns of HA-0.0ZnSi (a) and HA-2.0ZnSi (b) samples heated to different temperatures

4. CONCLUSIONS

In this work, it is shown that the mechanochemical method of synthesis makes it possible to obtain the zinc-silicate-substituted HA with an equal concentration of the substituent ions. The substitution limit is x = 1 mol per mole of HA. At the concentration of x = 2 mol, the formation of an amorphous non-crystallized phase together with HA-ZnSi phase is observed. The obviously high concentration of dopants makes it difficult to form the apatite structure.

It was found that the introduction of substituent ions with a concentration of up to 0.6 mol leads to a decrease in the lattice parameters, and at the higher concentration of dopants, an increase in the parameters occurs. The temperature treatment of the synthesized samples revealed a change in the dynamics of the parameters behavior, which indicates a structural transformations occurring in the crystal lattice. In this case, the appearance of a new absorption band is observed in the FTIR spectra, which indicates the formation of a new chemical bond. Explaining these transformations requires further more detailed research to be conducted in the future.

The obtained compounds can be promising for the medical purposes, for example, as a material for filling bone defects due to the beneficial properties of doped ions.

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